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Juvenile Hormone-Like Effects of a Thiazolylurea on Lepidopteran Larvae

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Heliothis virescens maintained on a diet containing 50 ppm of thiazolylurea (5-[[[2-thiazolylamino]amino]carbonyl]-1,3-benzenedicarboxylic acid dimethyl ester) showed symptoms of juvenile hormone overdose in the last larval instar. Increases in development time and weight, as well as larval color changes, were similar in animals fed thiazolylurea or topically treated with the juvenile hormone mimic methoprene. The juvenile hormone esterase titer profile in hemolymph of animals fed thiazolylurea was much broader than in controls, and peaked 2 days later than in controls; the premolt ecdysteroid peak in hemolymph of animals fed thiazolylurea appeared 2 days earlier than in controls. These events are characteristic of high hemolymph JH titers throughout the last larval instar.

Key words: juvenile hormone analog, insect development

INTRODUCTION

Recently we reported the effects of 5-[[[2-thiazolylamino]carbonyl]amino]-1,3 benzenedicarboxylic acid dimethyl ester (hereafter referred to as thiazolylurea) and related compounds on the growth and development of two lepidopteran species [1]. Animals reared on diet containing thiazolylurea were heavier, took longer to develop than controls, and showed other developmental abnormalities [1]. These effects were typical for insects treated with excess juvenile hormone or juvenile-hormone mimics [2]. However, thiazolylurea (Fig. 1) lacks the classical terpenoid structure of the natural JHs* or many of their successful mimics [3]. This work was undertaken to determine some of the specific endocrine changes elicited by thiazolylurea and to better understand its mode of action.

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*Abbreviations used: EPPAT = O-ethyl-S-phenyl phosphoramidothiolate; JH = juvenile hormone; JHE = juvenile hormone esterase; LD = long day, 16 h light, 8 h dark photoperiodic regimen; PTTH = prothoracicotropic hormone; SD = short day, 8 h light, 16 h dark photoperiodic regimen.

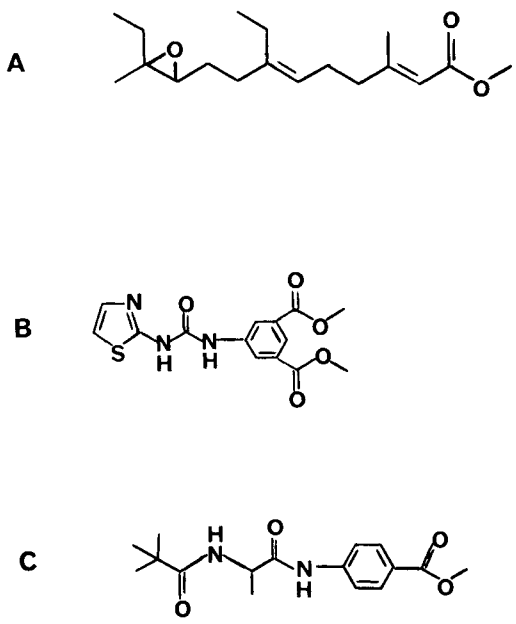

Fig. 1. Comparison of the structures of JH-active compounds. A: JH-I. B: Thiazolylurea. C: Ethyl pivaloyl-L-alanyl-*p*-aminobenzoate.

MATERIALS AND METHODS

Chemicals

Thiazolylurea, 5-[[[2-thiazolylamino]carbonyl]amino]-1,3-benezenedicarboxylic acid dimethyl ester, was synthesized according to the method of DeMilo et al. [1]. Its purity, estimated by TLC, HPLC, and elemental analyses, was > 98%. Methoprene was a gift by Zoecon Research Institute, Palo Alto, CA, and was 95% pure.

Experimental Insects

Heliothis virescens pupae were obtained from the Southern Field Crops Insect Management Laboratory (Stoneville, MS) as pupae. The pupae developed into adults, which mated and laid eggs. Resulting hatched larvae were reared in individual cups on modified Adkisson-Vanderzant medium [4]. Insects were observed during development and staged daily during the last larval instar, as in Loeb and Hayes [5]; N: newly molted 5th instar, S: slim, P: puffy, D: digging, B: buried, and Pu: pupa. Stage subscripts indicate the number of days at each stage. Thirty degree C controls, thiazolylurea-fed, methoprene- and acetone-treated insects were maintained in the regimen that ordinarily induces continual development (30°C, LD). A second set of controls were maintained in the diapause-inducing regimen of 20°C, SD [5].

Thiazolylurea Treatment

Thiazolylurea was dissolved in 1 ml of warm ethanol and mixed well with a warm, liquid diet to effect a concentration of 50 ppm (25 mg/500 ml diet) or

100 ppm (50 mp/500 ml diet); and approximately 0.5 oz was dispensed to each 1 oz diet cup. One newly hatched larva was placed in each cup.

The black mutant strain of *Manduca sexta* (obtained from L. Riddiford, Seattle, WA) was reared on artificial diet [6]. Diet containing 50 ppm of thiazolylurea was prepared as described above for *H. virescens* diet. Seven newly molted 4th-instar larvae were added to each batch of untreated and experimental diet and observed at the molt from the 4th to 5th instar, 5 days later. Thiazolylurea was also applied topically to the dorsal surfaces of newly molted 4th-instar black mutant larvae maintained on normal diet. Each dose was applied to three larvae in 0, 0.5, 5, and 50 ng amounts, dissolved in tetrahydrofuran:water (4:1). Observations for color changes were made at the next molt, 5 days later.

Larval and Pupal Weight Determination

Newly ecdysed last-instar *H. virescens* larvae were briefly rinsed with water to remove diet and frass, blotted dry, and weighed. The procedure was repeated once a day until pupation. The sample size was 12–17 insects.

JHE Titer Determination

Hemolymph oozing from a cut proleg was collected on a square of parafilm resting on ice. Ten microliters of hemolymph was taken up in a calibrated capillary tube and was blown into 1 ml of 20 mM sodium phosphate buffer (pH 7.4) containing 0.01% phenylthiourea. JHE activity was monitored by the partition assay of Hammock and Sparks [7] with 10-[^{3}H](N) JHI (New England Nuclear, Boston, MA). All rates are reported as JH-I hydrolysis/min/ml hemolymph. Addition of EPPAT (Courtesy of Philip McGee, Shell Oil Co., Houston, TX) (1 mM in phosphate buffer), a JHE inhibitor [7], was added to one set of hemolymph samples; non-inhibited replicates were also analyzed. Lack of hydrolysis of [^{3}H]JH-I in the set of samples containing EPPAT indicated that JH hydrolysis in our system resulted primarily from JHE activity.

Topical Application of Methoprene

The highest concentration of an acetone solution of methoprene applied topically that would allow approximately 75% survival of *H. virescens* larvae was 0.9 μg/μl. One microliter of this solution was delivered daily to the dorsal surfaces of larvae, from the 2nd instar to pupal ecdysis. Control larvae were treated similarly with 1 μl of acetone. Thirty-six experimental and 36 control larvae were treated, staged, and observed daily until pupation. Insects were weighed at pupation. Selected larvae were bled at day 2 (D2) of the last stadium; hemolymph was analyzed for JHE.

Ecdysteroid Titer Determination

H. virescens were rinsed and dried and hemolymph was collected, as described above. Two to five microliter samples of hemolymph were added to 500 μl of 75% methanol. Three to eight insects from control and treated groups were sampled throughout the last instar. After centrifugation at 1,500*g*, supernatants were removed, dried, and subjected to RIA for ecdysteroid determination [8,9]. Anti-ecdysteroid antibody had been prepared against a hemisuccinate derivative of ecdysone at the C-22 hydroxyl group. Tritiated ecdysone (63.5 Ci/mmol;

New England Nuclear, Boston, MA) was used as the ecdysteroid radioligand; 20-hydroxyecdysone (Calbiochem, La Jolla, CA) was used to construct the standard curve for each analysis. Samples were counted with a Beckman LS3801 scintillation counter.

Statistical Treatment of Data

Data for pupal weights and maximum JHE titers were analyzed for statistical differences by one-way analysis of variance followed by means separation using *t*-tests, which accommodated variable sample sizes. The experimentwise error rate (0.05) was controlled by adjusting the comparisonwise error rate ($\alpha = 0.008$) by the Bonferroni approach.

RESULTS

Development, Weight, and Color Changes in *H. virescens*

When *H. virescens* larvae (n = 12) were fed diet containing 100 ppm of thiazolylurea, only 25% of the insects pupated normally. The remainder either died as larvae (50%) or molted to larval-pupal intermediates (25%). Because of the severity of effects at 100 ppm, most of the data presented in this work was obtained with larvae fed on diet containing 50 ppm thiazolylurea; 95% of animals fed 50 ppm thiazolylurea diet developed into functional adults.

Neither thiazolylurea nor methoprene exerted noticeable effects on development time of *H. virescens* through four larval instars. The 5th (last) larval instar was, however, greatly affected. Larvae fed diet containing 50 ppm thiazolylurea took twice as long (approximately 14 days) to complete the last stadium as did larvae fed control diet (approximately 7 days) (Fig. 2A,C). Surprisingly, the extension of development time in thiazolylurea-fed last-instar larvae maintained at 30°C LD approximated the development time normally observed in last instar larvae maintained under diapause-inducing conditions [5] at 20°C SD (Fig. 2B). Daily topical application of the JH analog, methoprene, extended the last stadium, but to a lesser degree than did thiazolylurea (7–10 days) at 30°C LD. Acetone treatment was somewhat toxic, allowing only 63%

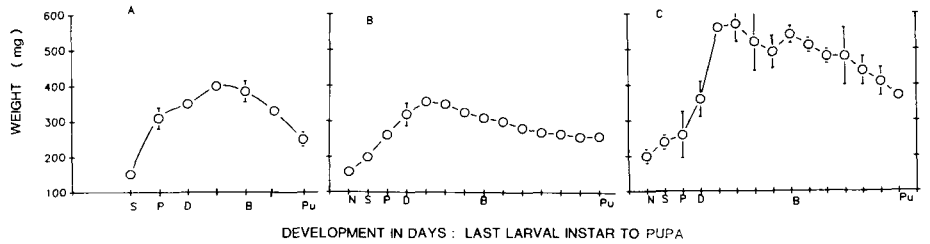

Fig. 2. Weight changes throughout the last larval instar of *Heliothis virescens*. **A**: 30°C LD controls. **B**: 20°C SD controls. **C**: 30°C LD larvae fed diet containing 50 ppm thiazolylurea. N = newly molted 5th-instar larva; S = slender feeding larva; P = puffy non-feeding larva; D = digging, feeding larva; B = buried larva to pre-pupa, Pu = pupa. Each tick on the *x* axis represents 1 larval day. Data points represent mean weights (mg) of 12–17 insects; bars indicate standard errors of the means. The absence of bars at any point indicates that the SEM was smaller than the symbol used.

of acetone-treated insects to pupate. However, only 36% of acetone-methoprene-treated insects pupated. All pupae eclosed as adults.

Larvae fed thiazolylurea or treated daily with methoprene were only slightly heavier than were controls up to the 4th instar. In the last instar, however, differences were easily seen (Fig. 2C). The maximum weight of all control last instar larvae was 350–400 mg, whereas that of insects fed thiazolylurea averaged 570 mg, or 1.4–1.6 times greater (Fig. 2). Weights of control pupae (Table 1) maintained at 30°C LD or 20°C SD, as well as controls treated topically with acetone, were statistically equal. Pupae resulting from thiazolylurea or methoprene treatment were 1.4 and 1.3 times heavier, respectively, than were controls (Table 1). Weights of both treated groups were statistically different from the control groups and each other.

Last instar larvae, reared under either regimen as controls, were generally green or purplish, with a small red or orange spot in each abdominal segment. Animals fed thiazolylurea tended to be paler green with large lavender-colored areas in each abdominal segment; insects treated with methoprene tended to be pale green with large rose-colored areas in each abdominal segment; insects treated with methoprene tended to be pale green with large rose-colored areas in each abdominal segment.

JHE Titers in *H. virescens*

JHE titers throughout the last larval stadium are shown in Figure 3. In both 30°C and 20°C control populations, peak titers occurred on the second day of the digging stage (D2); titers fell precipitously between the last day of digging and the first day of the buried stage (B1). Because of a slow decline in titer during the late digging period, the JHE titer curve was broader for 20°C controls than for 30°C controls. In contrast, JHE titers in thiazolylurea-fed larvae rose sharply at the puffy stage, earlier than in the control populations, but maintained a high plateau until B_2, after which the titer fell, but not to the low values observed in control populations. Peak JHE titers were statistically the same in 20°C SD controls, thiazolylurea-fed and methoprene-treated larvae; those for 30°C controls and acetone-treated 30°C controls were much lower (Table 2) and statistically different from the other groups.

TABLE 1. Pupal Weights After Treatment of *H. virescens* Larvae

Treatment	n start	n pupated	Weight (mg ± SEM)
Control, 30°C LD	58	55	250 ± 20
Control, 20°C SD	12	11	257 ± 7
Thiazolylurea 50 ppm in diet 30°C LD	40	38	340 ± 4
Methoprene (0.9 μg/μl) in acetone, topically applied daily 30°C LD	36	13	334 ± 14
Acetone topically applied daily 30°C LD	36	23	246 ± 10

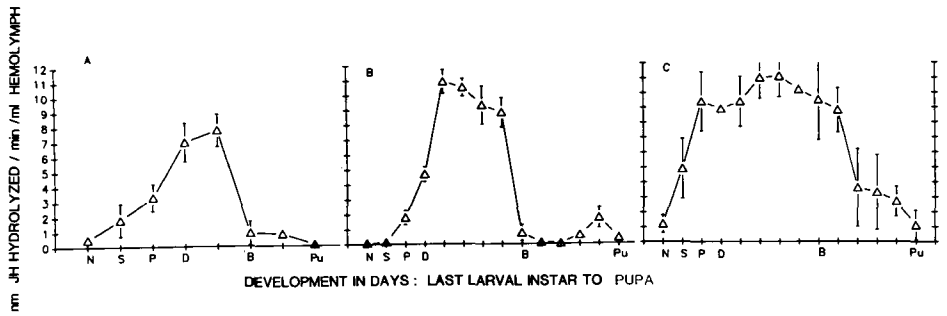

Fig. 3. JHE titers throughout the last larval instar of *H. virescens*. **A**: 30°C LD controls. **B**: 20°C SD controls. **C**: 30°D LD larvae fed diet containing 50 ppm thiazolylurea. Stages on *x* axis as in Figure 2. Data pionts represent mean titers determined from hemolymph of at least five larvae; bars indicate standard errors of the means. The absence of bars at any point indicates that the SEM was smaller than the symbol used.

Ecdysteroid Titers in *H. virescens*

Despite differences in temperature and photoperiod regimens, the early last-instar plateaus in hemolymph ecdysteroid titer occurred at the same times in control populations (S and D_2) (Fig. 4A,B). The pre-molt rise in titer at B_2 is drawn extended to the new pupal stage in Figure 4A, although not enough points were taken to determine whether there was indeed a drop in titer prior to pupation as *M. sexta* other Lepidoptera [10]. In control 20°C SD larvae, the pre-molt peak is noted at B_4. In contrast, peaks early in the last stadium in thiazolylurea-fed larvae (Fig. 4C) occurred 1 day later than in controls, at P and D_3, whereas the later peak occurred a day earlier than in 20°C controls at B_3. Maximum titers of ecdysteroid in hemolymph at the large pre-molt peak were 1,741 ± 312 pg/μl in 30°C LD control larvae, 680 ± 100 pg/μl in 20°C SD control larvae, and 935 ± 199 pg/μl on day B_3 in insects fed thiazolylurea; they were higher in newly ecdysed pupae of both 30°C control and thiazolylurea-treated insects (4,868 ± 435 and 5,898 ± 231 pg/μl, respectively). Hemolymph titers in 20°C control pupae in diapause were considerably lower [11].

TABLE 2. Maximum Juvenile Hormone Esterase Levels

Treatment	n	Last instar stage when peak titer appears	nmol JH-I hydrolyzed ml hemolymph/min
30°C LD (control)	10	D_2	7.8 ± 1.2
20°C SC (control)	10	D_2	10.9 ± 0.8
Thiazolylurea (50 ppm in diet) 30°C LD	10	D_3–D_4	11.0 ± 1.4
Methoprene + (0.9 μg/μl in acetone topically applied 30°C LD	5	D_2	11.3 ± 0.3
Acetone topically applied 30°C LD	2	D_2	2.32

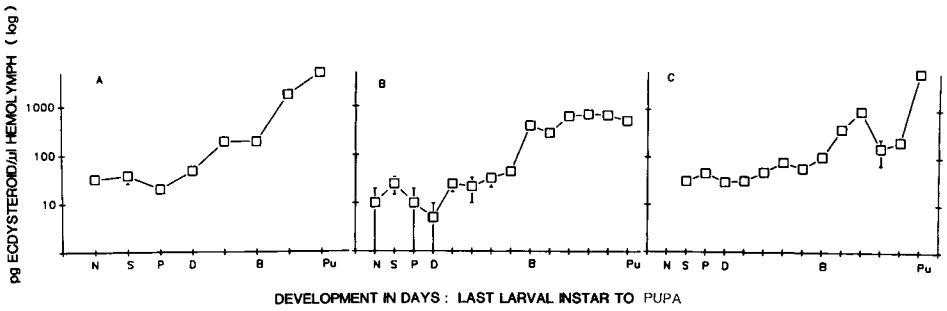

Fig. 4. Ecdysteroid titers throughout the last larval instar of *H. virescens*. **A**: 30°C LD controls. **B**: 20°C SD controls. **C**: 30°C LD larvae fed diet containing 50 ppm thiazolylurea. Data points represent mean titers determined from hemolymph of three to eight larvae; bars indicate standard errors of the means. B is replotted data [11]. Note that the *y* axis is the log of the hemolymph ecdysteroid titer. The absence of bars at any point indicates that the SEM was smaller than the symbol used.

Effects of Thiazolylurea on Black Mutant *M. sexta* Larvae

Black mutant larvae fed diet containing 50 ppm thiazolylurea for five days during the 3rd and 4th instar did not exhibit any color changes and topical application of up to 50 ng of thiazolylurea had no effect.

DISCUSSION

In Lepidoptera, the titer of JH is generally high at the beginning of the last larval stadium, drops abruptly to a level approaching zero near the middle of the instar, and rises slightly again just prior to pupation [2,10]. A burst of PTTH elicits a small burst of ecdysteroid synthesis by the prothoracic glands just after the drop in JH titer; another burst of PTTH release induces the later and much greater production of ecdysteroid shortly before the pupal molt [2,10]. High titers of JH at the beginning of the stadium tend to inhibit the release of PTTH and render the prothoracic glands incompetent to respond to PTTH. Thus, the neuroendocrine stimulus for secretion of the first small burst of ecdysone from the prothoracic glands normally occurs after the drop in JH titer (see [10]). It is believed that the first ecdysteroid peak leads to commitment of larval tissues to their pupal fates at the next molt [2,10,12–14]. Absence or delay of the small ecdysteroid peak leads to extension of the larval feeding period and concommitant increase in larval weight. If high JH titers are present at the time of commitment, larval-pupal intermediates, supernumerary larval molts, or loss of the ability to molt may result [12,14,15]. In contrast, excess JH administered after pupal commitment tends to accelerate pupation by stimulating early release of the second PTTH pulse, thereby inducing prothoracic glands to synthesize premolt ecdysteroid sooner than in control populations [10,12–14].

Treatment of *H. virescens* with thiazolylurea induced the JH-like effects of increased last-instar larval development time and the high larval and pupal weights. The highest dose (100 ppm of thiazolylurea) in the diet induced larval-

pupal intermediates at the larval-pupal molt in 25% of the population, and prevented the pupal molt in 50% of the treated insects. Similar results [1] were obtained by feeding thiazolylurea at 50 ppm to larvae of *Spodoptera frugiperda* and *Plodia interpunctella;* larval-pupal intermediate forms were also induced in these species. Topical application of the JH mimic, methoprene, to *H. virescens* larvae at concentrations yielding optimum survivability did not have as radical an effect on development time as it did on the weights of surviving larvae and pupae. However, it has been reported that methoprene greatly prolonged larval development of *Heliothis armigera* when fed or topically applied and induced larval-pupal intermediates [16]. Similar effects were observed in other lepidopteran species when JH or JH analogues were topically applied [12,13, 17–19]. The color patterns that we observed in larvae fed thiazolylurea were similar to those induced by methoprene, further emphasizing the similarity of the effects of thiazolylurea and JH analogue.

Work with another noctuid, *Trichoplusia ni* [20,21] as well as *M. sexta* [22,23] has demonstrated that hemolymph titers of JHE in last instar larvae are closely correlated to hemolymph titers of JH. Peaks of JHE often denote the end of a JH presence. This usually occurs twice in the last larval instar, in mid-instar, just prior to the first burst of PTTH, and again just prior to pupation [20–23]. Furthermore, production of JHE can be stimulated by either endogenous [21] or exogenous JH early as well as late in the last larval instar, even at stages normally devoid of JHE [20–23]. Further, in vitro inhibition of JHE will cause abnormally high titers of JH (Jones et al., unpublished data).

Neither thiazolylurea nor methoprene affected weight or timing of development to any appreciable extent in the first 4 larval stadia of *H. virescens.* Since JH and JH mimics have little or no effect on JHE titers in instars 1–4 in *T. ni* and *M. sexta* [20,22], this result is not surprising. The last instar of *H. virescens* was most affected by thiazolylurea and methoprene in this study. These data are similar to reports in the literature [20–23] that relate induced high titers of JHE in the hemolymph of last-instar larvae to abnormally high titers of JH or JH mimics.

Our data for *H. virescens* indicate a sharp JHE peak in the hemolymph of 30°C control larvae at D_2 (stage) of the last instar. Although the JHE titer rose as rapidly at D_2 in 20°C diapause-bound control larvae, it remained high for several days and declined when larvae entered the B stage 3 days later. These data suggest that diapause-bound larvae of *H. virescens* may be exposed to circulating JH for a longer period than are non-diapause-bound insects, as reported for other species of lepidoptera [18,24]. The JHE profile for last-instar larvae of *H. virescens* fed thiazolylurea was even broader than that for 20°C controls, suggesting early onset and long-lasting JH titers in the hemolymph of these insects. High JH titers in the hemolymph early in the instar can cause a delay in initial PTTH secretion and a consequent delay in the ecdysteroid programming peak [10]. The result of these perturbations is an acceleration of early last-instar growth and prolongation of the instar, as observed in *H. virescens* fed thiazolylurea. Peak titers of JHE were similar in 20°C controls and methoprene- and thiazolylurea-treated *H. virescens,* suggesting high titers of JH or JH agonist in the hemolymph of each of these populations.

In insects fed thiazolylurea, the early last-instar ecdysteroid peaks appeared

1 day later than in 30°C controls, whereas the late-instar peak appeared a day earlier than in 20°C controls. This is consistent with current theory [2,10,25] that high JH levels early in the instar delay PTTH release, and thus ecdysteroid secretion, whereas high JH levels late in the last instar accelerate PTTH release and induce an early premolt ecdysteroid peak. High JH levels late in the last stadium tend to induce release of a fat body trophic factor, which further increases the amount of ecdysteroid secreted by the prothoracic glands [25,26]. Possibly, the high ecdysteroid titers observed in the hemolymph of newly eclosed pupae from the thiazolylurea-fed population were a response to high fat body factor titer elicited by the apparent high JH titer.

Thiazolylurea did not act like JH in causing black mutant *M. sexta* larvae to revert to normal blue-green color when fed or topically applied [27]. However, *H. virescens* as well as *S. frugiperda* and *P. punctella* [1] ingested the compound from the 1st larval instar to the last, whereas *M. sexta* were treated for relatively short periods and may not have received comparable effective doses. On the other hand, *Musca domestica* fed 10 and 50 ppm levels of thiazolylurea throughout their lifetime were not outwardly affected [1]. Thiazolylurea was administered in most cases as part of the larval diet. It is possible, therefore, that the JH activity attributed to thiazolylurea is actually due to a metabolite resorbed in some species but not in others. Alternatively, it is possible that the action of thiazolylurea is species specific or that the metabolism of thiazolylurea differs in different species. This problem needs further exploration.

Although the endocrine effects of thiazolylurea are similar to those of JH, the structure of this compound is markedly unlike JH or any of its classically structured terpenoid mimics. However, some structural resemblance can be seen between thiazolylurea and certain nonterpenoid peptidic alkyl *p*-aminobenzoate juvenoids [3,28]. The structure of ethyl pivaloyl-L-alanyl-*p*-aminobenzoate, a typical representative of the *p*-aminobenzoate juvenoids, is shown in Figure 1C; its action is limited to hemipterans of the family Pyrrhocoridae [3,28]. Since all of these compounds induced JH-like effects, they may act indirectly by mimicking the action of the stimulatory neuropeptide, allatotropin [29–31] and thus accelerate synthesis of JH by the corpora allata. Alternatively, they may act directly by binding to receptors for JH in each cell.

LITERATURE CITED

1. DeMilo AB, Loeb MJ, Redfern RE, Borkovec AB, Hayes DK: Thiazolylureas: Effects on larval growth and development in the fall armyworm and tobacco budworm. J Agric Food Chem (in press).
2. Riddiford LM: Interaction of ecdysteroids and juvenile hormone in the regulation of larval growth and metamorphosis of the tobacco hornworm. In: Progress in Ecdysone Research. Hoffman JA, ed. Elseiver/North-Holland Biomedical Press, Amsterdam, pp 409–430 (1980).
3. Henrick CA: Juvenile hormone analogs: Structure-activity relationships. In: Insecticide Mode of Action. Coats JR, ed. Academic Press, New York, 315–402 (1982).
4. Adkisson PL, Vanderzant ES, Bull DL, Allison WE: A wheat germ medium for rearing the pink bollworm. J Econ Entomol *53*, 193 (1960).
5. Loeb MJ, Hayes DK: Critical periods in the regulation of the pupal molt of the tobacco budworm, *Heliothis virescens*. Ann Ent Soc Am *73*, 679 (1980).
6. Yamamoto RT: Mass rearing of the tobacco hornworm. II: Larval rearing and pupation. J Econ Entomol *62*, 1427 (1969).

7. Hammock BD, Sparks TC: A rapid assay for insect juvenile hormone esterase activity. Anal Biochem *82*, 573 (1977).
8. Borst DW, O'Connor JD: Arthropod molting hormone: Radioimmune assay. Science *178*, 418 (1972).
9. Bollenbacher WE, Vedekis WV, Gilbert LI, O'Conner JD: Ecdysone titers and prothoracic gland activity during the larval-pupal development of *Manduca sexta*. Dev Biol *44*, 46 (1975).
10. Bollenbacher WE: The interendocrine regulation of larval-pupal development in the Tobacco Hornworm, *Manduca sexta*. J Insect Physiol *34*, 941 (1988).
11. Loeb MJ: Diapause and development in the tobacco budworm, *Heliothis virescens:* A comparision of hemolymph ecdysteroid titers. J Insect Physiol *28*, 667 (1982).
12. Cymborowski B, Stolarz G: The role of juvenile hormone during larval-pupal transformation of *Spodoptera littoralis:* Switchover in the sensitivity of the prothoracic gland to juvenile hormone. J Insect Physiol *25*, 939 (1979).
13. Hiruma K: Factors affecting change in sensitivity of prothoracic glands to juvenile hormone in *Mamestra brassicae*. J Insect Physiol *28*, 193 (1982).
14. Rountree DB, Bollenbacher W: The release of the prothoracicotropic hormone in the tobacco hornworm, *Manduca sexta*, is controlled intrinsically by juvenile hormone. J Exp Biol *120*, 41 (1986).
15. Hiruma K: Regulation of prothoracicotropic hormone release by juvenile hormone in the penultimate and last instar larvae of *Mamestra brassicae*. Gen Comp Encodrinol *63*, 201 (1986).
16. Fytizas E: Influence due mode de traitement aux analogues de l'hormone juvenile sur l'apparition des effects. Med Fac Landbouww Rijksuniv Gent *42*, 1261 (1977).
17. Riddiford LM: Juvenile hormone in relation to the larval-pupal transformation of the Cecropia silkworm. Biol Bull *142*, 310 (1972).
18. Podoler H, Klein M, Hadar E, Applebaum SW: Physiological effects of a juvenile hormone analog on larvae of *Spodoptera littoralis*. Entomol Exp Appl *39*, 197 (1985).
19. Hatakoshi M, Nakayama I, Riddiford LM: The induction of an imperfect supernumerary larval molt by juvenile hormone analogues in *Manduca sexta*. J Insect Physiol *34*, 373 (1988).
20. Sparks TC, Hammock BD: Induction and regulation of juvenile hormone esterases during the last larval instar of the cabbage looper, *Trichoplusia ni*. J Insect Physiol *25*, 551 (1979a).
21. Jones G, Hammock BD: Pre-pupal regulation of juvenile hormone esterase through direct induction by juvenile hormone. J Insect Physiol *29*, 471 (1983).
22. Sparks TC, Hammock BD, Riddiford LM: The hemolymph juvenile hormone esterase of *Manduca sexta* (L.)—Inhibition and regulation. Insect Biochem *13*, 529 (1983).
23. Venkatech K, Roe RM: The role of a juvenile hormone and brain factor(s) in the regulation of plasma juvenile hormone esterase activity during the last larval stadium of the tobacco hornworm, *Manduca sexta*. J Insect Physiol *34*, 415 (1988).
24. Sieber R, Benz G: The hormonal regulation of the larval diapause in the codling moth *Laspeyresia pomonella* (Lep. Tortricidae). J Insect Physiol *26*, 213 (1980).
25. Watson RD, Ciancio MJ, Gunner WP, Gilbert LI, Bollenbacher WE: Regulation of insect prothoracic glands: Existence of a hemolymph stimulatory factor in *Manduca sexta*. J Insect Physiol *31*, 487 (1985).
26. Gruetzmacher MC, Gilbert LI, Granger NA, Goodman W, Bollenbacher WE: The effect of juvenile hormone on prothoracic gland function during the larval-pupal development of *Manduca sexta:* An *in situ* and *in vitro* analysis. J Insect Physiol *30*, 331 (1984).
27. Fain MT, Riddiford LM: Juvenile hormone titers in the hemolymph during late larval development of the tobacco hornworm, *Manduca sexta* (L.). Biol Bull *149*, 506 (1975).
28. Slama K, Romanuk M, Sorm F: Insect Hormones and Bioanalogues. Springer-Verlag, New York, pp 1–477 (1974).
29. Granger NA, Sehnal F: Regulation of larval corpora allata in *Galleria mellonella*. Nature *251*, 415 (1974).
30. Bogus MI, Cymborowski B: Induction of supernumerary moults in *Galleria mellonella:* Evidence for an allatotropic function of the brain. J Insect Physiol *30*, 557 (1984).
31. Gadot M, Applebaum SW: Rapid in vitro activation of corpora allata by extracted locust brain allatropic factor. Arch Insect Biochem Physiol *2*, 117 (1985).